ISIS-4789 PATENT

hexane:ethylacetate (25:75) gives the title compound.

#### **EXAMPLE 74**

# 5'-Dimethoxytrityl-2'-O-(2-methoxyphenyl)-5-methyluridine-3'-O-succinate

[0256] The 5'-protected nucleoside from Example 73 is treated with 2 equivalents of succinic anhydride and 0.2 equivalents of 4-N,N-dimethylaminopyridine in pyridine. After 2 hours the pyridine is evaporated, the residue is dissolved in CH<sub>2</sub>Cl<sub>2</sub> and washed three times with 100 mL of 10% citric acid solution. The organic layer is dried over anhydrous MgSO<sub>4</sub> to give the desired succinate. The succinate is then attached to controlled pore glass (CPG) using established procedures (Pon, R.T., Solid phase supports for oligonucleotide synthesis, in *Protocols for Oligonucleotides and Analogs*, S. Agrawal (Ed.), Humana Press: Totawa, NJ, 1993, 465-496).

#### **EXAMPLE 75**

## 5'-Dimethoxytrityl-2'-O-(trans-2-methoxycyclohexyl)-5-methyl uridine

[0257] 2'-3'-O-Dibutylstannyl-5-methyl uridine (Wagner et al., J. Org. Chem., 1974, 39, 24) is alkylated with trans-2-methoxycyclohexyl tosylate at 70 °C in DMF. A 1:1 mixture of 2'-O- and 3'-O-(trans-2-methoxycyclohexyl)-5-methyluridine is obtained in this reaction. After evaporation of the DMF solvent, the crude mixture is dissolved in pyridine and treated with dimethoxytritylchloride (DMT-Cl) (1.5 equivalents). The resultant mixture is purified by silica gel flash column chromatography to give the title compound.

## **EXAMPLE 76**

5'-Dimethoxytrityl-2'-O-(trans-2-methoxycyclohexyl)-5-methyluridine-3'-O-(2-cyanoethyl-N,N-diisopropylamino) phosphoramidite

[0258] 5'-Dimethoxytrityl-2'-O-(trans-2-methoxycyclohexyl)-5-methyl uridine is phosphitylated according to the procedure described above to give the required phosphoramidite.

ISIS-4789 PATENT

#### **EXAMPLE 77**

# 5'-Dimethoxytrityl-2'-O-(trans-2-methoxycyclohexyl)-5-methyluridine-3'-O-(succinylamino) CPG

[0259] 5'-Dimethoxytrityl-2'-*O*-(*trans*-2-methoxycyclohexyl)-5-methyl uridine is succinylated and attached to controlled pore glass to give the solid support bound nucleoside.

#### **EXAMPLE 78**

#### trans-2-ureido-cyclohexanol

[0260] Trans-2-amino-cyclohexanol (Aldrich) is treated with triphosgene in methylene chloride (1/3 equivalent). To the resulting solution, excess ammonium hydroxide is added to give after work up the title compound.

#### **EXAMPLE 79**

## 2'-O-(trans-2-uriedo-cyclohexyl)-5-methyl uridine

[0261] Trans-2-uriedo-cyclohexanol (50 mmol) is added to a solution of borane in tetrahydrofuran (1 M, 10 mL, 10 mmol) while stirring in a 10 mL bomb. Hydrogen gas evolves as the reactant dissolves. O2,2'-Anhydro-5-methyluridine (5 mmol) and sodium bicarbonate (2.5 mg) are added to the bomb and sealed. Then it is heated to 140 for 72 hrs. The bomb is cooled to room temperature and opened. The crude material was worked up as illustrated above followed by purification by silica gel flash column chromatography to give the title compound.

## **EXAMPLE 80**

5'-O-(Dimethoxytrityl)-2'-O-(trans-2-uriedo-cyclohexyl) 3'-O-(2-cyanoethyl, N,N,-diisopropyl) uridine phosphoramidite

[0262] 2'-O-(trans-2-uriedo-cyclohexyl)-5-methyl uridine tritylated at the 5'-OH and phosphitylated at the 3'-OH following the procedures illustrated in example 2 to give the title compound.

ISIS-4789 PATENT

#### **EXAMPLE 81**

5'-O-dimethoxytrityl-2'-O-(trans-2-uriedo-cyclohexyl)-5-methyl-3'-O-(succinyl)-amino CPG uridine

[0263] 5'-O-dimethoxytrityl-2'-O-(trans-2-uriedo-cyclohexyl)-5-methyl uridine is succinylated and attached to CPG as illustrated above.

#### **EXAMPLE 82**

## 2'-O-(trans-2-methoxy-cyclohexyl) adenosine

[0264] Trans-2-methoxycyclopentanol, trans-2-methoxycylcohexanol, trans-2-methoxycyclopentyl tosylate and trans-2-methoxy-cyclohexyl tosylate are prepared according to reported procedures (Roberts, D.D., Hendrickson, W., J. Org. Chem., 1967, 34, 2415-2417; J. Org. Chem., 1997, 62, 1857-1859). A solution of adenosine (42.74 g, 0.16 mol) in dry dimethylformamide (800 mL) at 5 °C is treated with sodium hydride (8.24 g, 60% in oil prewashed thrice with hexanes, 0.21 mol). After stirring for 30 min, trans-2-methoxycyclohexyl tosylate (0.16 mol) is added over 20 minutes at 5 °C. The reaction is stirred at room temperature for 48 hours, then filtered through Celite. The filtrate is concentrated under reduced pressure followed by coevaporation with toluene (2x100 mL) to give the title compound.

#### **EXAMPLE 83**

## N<sup>6</sup>-Benzoyl-2'-O-(trans-2-methoxycyclohexyl) adenosine

[0265] A solution of 2'-O-(trans-2-methoxy-cyclohexyl) adenosine (0.056 mol) in pyridine (100 mL) is evaporated under reduced pressure to dryness. The residue is redissolved in pyridine (560 mL) and cooled in an ice water bath. Trimethylsilyl chloride (36.4 mL, 0.291 mol) is added and the reaction is stirred at 5 °C for 30 minutes. Benzoyl chloride (33.6 mL, 0.291 mol) is added and the reaction is allowed to warm to 25 °C for 2 hours and then cooled to 5 °C. The reaction is diluted with cold water (112 mL) and after stirring for 15 min, concentrated